

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

See notes

6. Budget Plan:

Renewal

Salary
Ext. & Lab. Supplies
Application For Research Grant

Overhead (13%)
Other (Travel)

\$28 R1

Date: October 6, 1955

1. Name of Investigator: George E. Moore, M.D., Ph.D.
2. Anticipated Duration of Work: F. Bock, M.S.

2. Title: Dr. Moore - Director, Roswell Park Memorial Institute, Deputy Treasurer,
Health Research Inc., Roswell Park Division
Mr. Bock - Senior Cancer Research Scientist

3. Institution: The program covered by this application. Animal quarters are available for mice and dogs, and Roswell Park Memorial Institute has a large mouse breeding colony so the 666 Elm Street or also is a convenient place for animal and laboratory supplies that at Buffalo 3, New York. Several storehouses at the Institute are available to store charges against the grant. Tissue preparation and pathology service is also available against the grant.

4. Project or Subject: Charge against the grant.
An investigation of the physiological effects of direct inhalation of tobacco smoke by laboratory animals.
In addition, two laboratory technicians, and two research assistants. Other members of the staff of the Institute have been generous in offering suggestions in their special fields.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

10. Additional information (including results of work to other projects and other sources of support):
I. Smoke inhalation - The major activity of the program has consisted of application of cigarette smoke directly to individual mice. In the animals which have been examined to date, we have observed no tumors of the bronchial epithelium. Strong A mice which were exposed to cigarette smoke over a period of five months did not show a high level of pulmonary adenomas.

During the coming year, it is planned to set up one additional series of Swiss mice in this experiment. On the basis of our experience with this strain of mice in the apparatus now being used, the number of cigarettes smoked per day will be increased from three to ten during the first two months. At these levels, losses from acute toxicity will be small. Animals currently on the experiment will be continued at the treatment levels that have already been set up for their respective groups.

Our experience with the design of a smoking chamber for larger rodents indicates that similar instruments will be most suitable. The use of inner cones is particularly desirable for rats inasmuch as it provides for continuous growth of the animals. Accordingly, smoking machines of size sufficient for rats and hamsters will be employed to test the effect of exposure to smoke upon these animals. Such an expansion of the experiment should do much to determine whether species specificity towards cigarette smoke carcinogenesis might be an important factor in experiment design. In this regard, the high species specificity towards the polycyclic hydrocarbons is noteworthy.

II. Studies of Crude Cigarette Smoke Condensate - Our evidence indicates that analysis of the effect of cigarette smoke condensate upon the sebaceous glands of

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mice provides a specific measure of the presence of polycyclic hydrocarbons. Within this class of compounds the relative carcinogenic activity against mouse skin matches the relative effect upon the sebaceous glands of mice. It seems probable that the materials which cause disappearance of these glands may also be responsible for the skin tumors observed in mice painted with cigarette smoke condensate.

Since species specificity is a striking characteristic in the response of not only rodents but also other mammals to skin painting by hydrocarbons, it would seem that the mouse is perhaps not the agent of choice in such analyses. Therefore, we propose to test a series of animals available to us, including rats, hamsters, guinea pigs, rabbits, dogs, and monkeys in order to determine the specificity of the response of the sebaceous glands to the application of crude cigarette smoke condensate. If whole mount technique proves suitable for animals with thick skins, the test may be extended to human subjects. The object of this phase of the investigation will be to determine the test animal of choice if differences exist.

The second phase will involve testing of various cigarettes for this general biological effect. It is planned to use three of the "American Smoking Machines" available from the Phipps and Bird Co. for this purpose. Using our present condensates, from a multiple smoking machine, we have found that the "tar" from only 40 cigarettes is sufficient for one test. Thus it becomes possible to test the material within hours of collection in order to minimize changes that might occur with storage.

This series of experiments should be sufficient to demonstrate whether there is species specificity towards the substance(s) in cigarette smoke condensate that lead to skin tumors in mice. Further, the experiments will demonstrate just how this property of smoke condensate varies with different methods of smoking, different tobaccos, and interposition of different filters or other methods of alteration of smoke composition.

III. Isolation of Fractions from Cigarette Smoke Condensate - With the development of the whole mount technique and use of serial dilution for measurement of activity against sebaceous glands, it was found possible to fractionate the smoke condensate systematically in an attempt to characterize the responsible compound(s) responsible for this effect. Preliminary fractionation led to the conclusion that counter-current distribution would lead to separation of the "active material" from the bulk of inactive residues. Subsequently, a 200 tube countercurrent distribution instrument was ordered by the State of New York for this project. Delivery is expected in December. With this equipment, it should be possible to concentrate the active material in quantities sufficient for bioassay with a minimum of treatment time.

IV. Studies of the Effects of Raw Tobacco in Various Diets - Our early experiments of 14 months duration have demonstrated that raw tobacco has no carcinogenic effect when fed at less than toxic levels to both mice and rats. An evaluation of the human experience of mouth and throat cancer among tobacco "chewers" shows that much of the positive correlation is observed in geographic areas where the general diet is poor. Accordingly, diets have been set up with deficiencies of vitamin A, thiamin, and riboflavin. Dried raw cigarette tobacco is added in level that, in our experience, is about 1/2 of the minimum lethal dose. In one group, tobacco extracted with acetone and petroleum ether is used to control the effect of added cellulose etc. in the diets. These experiments are underway and will be continued.

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BUDGET PLAN Dec. 1, 1955 - Nov. 30, 1956

6. EXPLANATION OF SALARIES AND PERMANENT EQUIPMENT:

Salaries

Mund (Senior Medical Technician)	\$3,730
Harris (Senior Animal Caretaker)	3,511
Mickens (Laboratory Worker)	2,746
Brailsford (Laboratory Worker)	2,598
Adams (Laboratory Worker)	2,598
Ramadhan (Laboratory Helper)	2,460
Bennett (Laboratory Helper)	<u>2,236</u>

Social Security (2%)	\$19,879.00
Workmen's Compensation (1.54%)	<u>397.58</u>
	<u>306.14</u>

20,582.72

Permanent Equipment

American Smoking Machine 3 @ \$1,250

3,750.00

PREDICATED BUDGETARY NEEDS -- Dec. 1, 1956 - Nov. 30, 1957

Salaries

Senior Medical Technician	1	\$3,900
Laboratory Worker	3	8,200
Laboratory Helper	1	<u>2,400</u>

Social Security and Workmen's Comp.

\$14,500
<u>500</u>

15,000

Expendable Supplies

2,250

Permanent Equipment

300

Travel

250

Overhead

<u>2,670</u>
\$20,470

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6. Budget Plan:

	Salaries	\$20,582.72
<i>Renewal</i>	Expendable Supplies	2,250.00
Application For:	Permanent Equipment	3,750.00
	Overhead (15%)	4,024.91
	Other (Travel)	250.00
	Total	\$30,857.63

1. Name of Investigator: George E. Moore, M.D., Ph.D.
7. Anticipated Duration of Work: 2 years

2. Title: 2 years
8. Facilities and Staff Available: Director, Roswell Park Memorial Institute, Deputy Director, Health Research Lab., Roswell Park Memorial Institute

The facilities available at the Roswell Park Memorial Institute are well adapted for the program covered by this application. Animal quarters are available for monkeys, dogs, and rodents of all types. The Institute maintains a large mouse breeding colony so that no purchases of mice is necessary. All general and laboratory supplies that are carried in the general storehouse of the Institute are available without charge against the grant. Tissue preparation is carried out by a service laboratory without charge against the grant.

The Institute personnel who work upon this project or directly related problems include Dr. George E. Moore, Mr. Fred G. Bock, Dr. Joseph DiPaolo, and in addition, two laboratory technicians, and five animal caretakers. Other members of the staff of the Institute have been generous in offering suggestions in their special fields.

5. Statement of Procedure (Use reverse side if additional space is needed)
10. Additional Information (Including relation of work to other projects and other sources of supply):

1. Cigarette Inhalation - The major activity of the program has consisted of application of cigarette smoke directly to individual mice. In the animals which have been exposed to date, we have observed no change of the bronchial epithelium. Strongly and mice which have been exposed to cigarette smoke over a period of five months did not show a high level of pulmonary adenoma.

During the coming year, it is planned to set up an additional series of mice in this experiment. On the basis of our experience with this strain of mice in the laboratory now being used, the number of cigarettes smoked per day will be increased from three to ten during the first two months. At these levels, losses from acute toxicity will be small. Animals currently on the experiment will be continued at the treatment levels that have already been set up for their respective groups.

Our experience with the design of a smoking chamber for larger rodents indicates that similar instruments will be soon available. The use of inner cones is particularly desirable for rats inasmuch as it provides for continuous growth of the animal. Accordingly, smoking machines of size sufficient for rats and hares will be employed to test the effect of exposure to smoke upon these animals. Such an exposure of the animals experiments should do much to determine whether species specificity towards cigarette smoke carcinogenesis might be an important factor in experimental design. In this regard, the signature of the Director of Project is noteworthy.

11. Studies of Crude Cigarette Smoke Condensates - Our evidence indicates that analysis of the effect of cigarette smoke condensates upon the labial glands of